INFLUENCING FACTORS ON THE PRODUCTION OF EXOPOLYSACCHARIDES BY *PSEUDOMONAS AERUGINOSA*

by

CHELBEE MARIE FARNEN

APPROVED:

Dr. Crosby W. Jones, Chair
Professor of Biology

Dr. Loren K. Ammerman,
Professor of Biology

Dr. Nicholas Negovetich,
Assistant Professor of Biology

May 3, 2016
Date Successfully Defended and
Approved by Advisory Committee

APPROVED:

Dr. Shirley M. Eoff
Director of the Honors Program

May 13, 2016

INFLUENCING FACTORS ON THE PRODUCTION OF EXOPOLYSACCHARIDES BY *PSEUDOMONAS AERUGINOSA*

A Thesis

Presented to the

Honors Program of

Angelo State University

In Partial Fulfillment of the

Requirements for Highest University Honors

BACHELOR OF SCIENCE

by

CHELBEE MARIE FARNEN

May 2016

Major: Biology

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Crosby W. Jones for his amazing influence and encouragement. He is my number one advocate and always knows the right thing to say to cheer me up when my research does not go as planned. I would also like to thank Dr. Shirley M. Eoff for her encouragement for me to complete this honors thesis and to never give up. I am very appreciative of the rest of my committee Dr. Loren K. Ammerman and Dr. Nicholas Negovetich for agreeing to help me complete this thesis. They have given up their own free time to edit my work and to attend my defense. I am grateful that I chose to attend Angelo State University and met all of these amazing mentors along the way who have encouraged my research and taught me valuable information in the classroom. Lastly, I would like to thank my friends and family who have unfailingly stayed by my side through this whole process. Without their encouragement and support I would have never been able to achieve my goal. Thank you everyone for this amazing experience.

ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen infecting patients with compromised immune systems, cystic fibrosis, or serious burns. These bacteria produce many virulence factors affecting these patients, but the one most responsible for morbidity and mortality in cystic fibrosis patients is a sticky, slimy substance called alginate. This component along with other exopolysaccharides is produced when the bacteria are grown in a liquid environment. It can be measured using a simple flow through method previously developed by the author. Using this method, experiments were performed providing the bacteria with a single carbon source to determine if certain carbon sources allow the bacteria to increase or decrease production of this virulence factor. Additional inorganic substances and mechanical manipulations were also tested to detect their effect on slime production. The bacteria grew well in media containing a single sugar carbon source, but failed to produce the alginate component when no amino acid or other nitrogen source was available. These results might be used to develop a hypothesis concerning bacterial signaling components and/or the chemical materials absolutely necessary for alginate production.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive genetic disorder in Caucasians (1). More than 33,000 people in the US and 70,000 worldwide are living with CF (CF foundation). Carriers of this disease, individuals having only one mutated gene, occur commonly with about 1 in 25 of the Caucasian population carrying one of many mutations, and when two carriers have a child there is a 25% chance this child will have CF (1). This disease greatly shortens the lifespan with the current median age in the United Kingdom being 41 years. The major cause of morbidity and mortality in CF patients is respiratory disease generally caused by secondary infection (1). The primary goal in treating these patients is to prevent the chronic infection caused by bacteria such as *Pseudomonas aeruginosa*. To achieve this, patients are given antipseudomonal antibiotics and additional agents to liquefy the thick respiratory mucus. The use of nebulizers allows for inhalation of the medications to treat the patient directly at the source. Other treatments involve loosening the mucus in the airways through physical techniques including pounding on the chest or aggressive breathing techniques that help to bring the mucus out of the lungs. The main goal of these treatments is to improve breathing and to prevent *Pseudomonas aeruginosa* from colonizing the lungs and causing chronic infection (1). The cost for this treatment averaged $40,037 per person per year in 2008 with the cost increasing over the lifetime as the disease progresses (2). With 33,000 people in the US needing this treatment, this disease plays a large role in the US healthcare costs. In addition, the constant treatment of these patients with antibiotics causes an increase in antibiotic resistance by PA as well as other commensal bacteria (3). These bacteria can cause nosocomial infections in immunocompromised

hospital patients, further increasing health care concerns and costs. The present research investigated influential factors on the production of bacterial components that aid in this chronic lung infection. If these processes can be stopped before the bacteria have the chance to initiate them, the threat of chronic infection could be thwarted. It is essential to understand the nature of *Pseudomonas aeruginosa* and its virulence factors to have a chance of controlling this chronic disease in CF patients. This research shed some light on some important factors that could decrease the pathogenesis of these bacteria.

Pseudomonas aeruginosa (PA) (Fig. 1) is an opportunistic pathogenic bacterium that most affects those with a compromised immune system. Cystic fibrosis (CF) and burn patients are among those with the most severe prognosis once infected, and this bacterium is the number one cause of morbidity and mortality in individuals with cystic fibrosis (4). It is able to evade many treatments and to exclusively colonize the lungs of CF patients due to its wide range of virulence factors including pyocyanin, alginate, biofilm and others. These factors each play a role in the infection and, combined, these elements cause deadly consequences (3,4,5,6). The present research investigated minimal media containing various components and mechanical treatments to determine factors that might cause an increase or a decrease in alginate production by this species of bacteria.

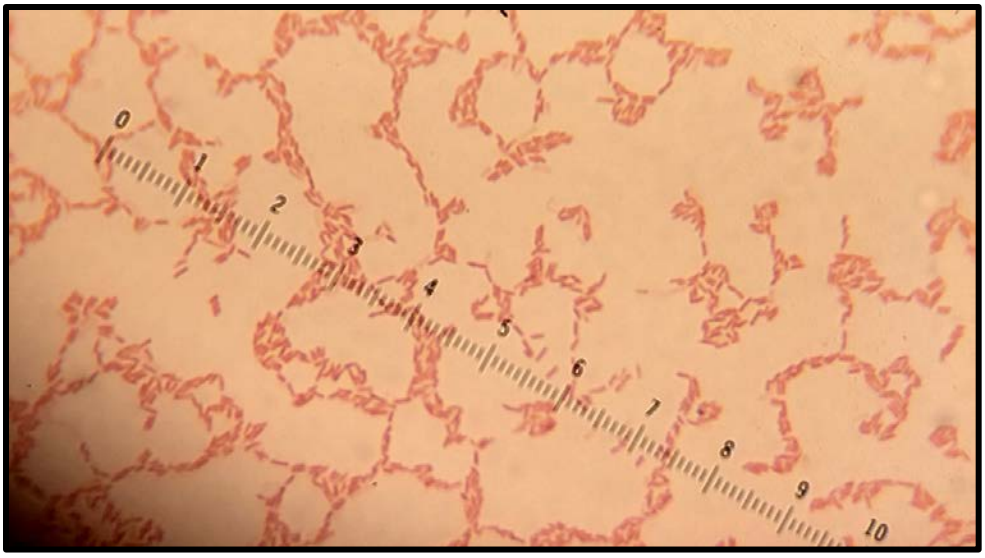


FIG 1 *Pseudomonas aeruginosa* are small gram-negative rods as seen in this gram stain.

The author first developed the method used in this research over the course of a year due to the lack of quantitative methods in the literature that were economically feasible, simple, and quick. The most common quantitative test for measuring alginate specifically involves precipitating the compound out of solution, then drying and weighing steps (7,9). This procedure can take many days and can only measure one aspect of the slime produced. The flow through method in this paper allows for all slime components that contribute to the overall viscosity to be measured with accuracy and reproducibility. The production of the finalized method used in this research took many trial and error steps to accomplish and was at first inaccurate due to bubble formation when the liquid was poured into the device. This was counteracted by the introduction of a stopper, which allows the researcher to remove any bubbles formed before the test is conducted. Determining whether statistically similar results were obtained from multiple trials when all experimental conditions were kept the same established the reproducibility of the method. Once reproducibility was confirmed the method could be used for further testing of PA slime production.

Virulence factors of PA that influence the amount of slime, a collective term referring to the thickening of the broths when certain extracellular components are produced, include pyocyanin, alginate, and biofilm. Pyocyanin is a redox active compound that creates chaos in the respiratory epithelial cells of CF patients and leads to the increased ability for colonization by this bacterial species (4). This chemical has the ability to carry electrons for the bacteria. It produces a blue color characteristic of these bacteria that results when it donates an electron to oxygen forming a superoxide anion. Pyocyanin is used as part of a signaling mechanism that helps determine the morphology of the PA colonies and also triggers multiple cellular changes in the respiratory tract.

Another factor commonly produced by these bacteria is biofilm. A biofilm is defined as a collection of polysaccharide components that protectively encase the bacteria and allow for the development of a well organized, thriving community (5). This layer protects the bacteria from immune cells and antibiotics and allows them to proliferate even if the upper layers are temporarily destroyed.

Alginate is a unique substance that is the focus of this research. It is an extracellular polysaccharide component composed of β-D-mannuronate and α-L-guluronate that, similar to the biofilm, allows the bacteria to evade immunological and medical destruction, and when produced in the lungs, can cause a multitude of breathing problems because oxygen diffusion into the alveoli is greatly inhibited (3,4). The production, modification, and excretion of this compound is quite complex involving thirteen different proteins produced from several genes within the same region of the large chromosome of PA (6,8,9). It has been shown that PA uses environmental signals to initiate slime production, and to allocate carbon resources into the different cellular polysaccharide components including alginate,

biofilm, and lipopolysaccharide (LPS) (5). Understanding the environmental cues that influence the production of each of these components is important in understanding how to fight these bacteria and eliminate them.

In this study, minimal broths were used to test differences in alginate production. These broths contained inorganic salts and one carbon source including asparagine, citrate, glucose, or mannose in a one or three percent concentration. In addition, some cultures were shaken for aeration purposes and some had additional components added while incubated to determine the effects of specific factors such as calcium. The asparagine broth is known to cause an abundance of pyocyanin production that can clearly be seen using a black light to elicit a blue fluorescence. Because pyocyanin is a known signaling molecule that initiates certain mucoid mechanisms (4), including asparagine broth in the study helped determine if pyocyanin could influence the production of alginate in these bacteria. A minimal citrate broth was tested because previous studies have demonstrated a correlation between citrate and the regulation of alginate secretion and because of its ability to chelate calcium ions (6). Glucose was an obvious choice for this research because it is a simple sugar that can be used by the bacteria and converted into many different compounds. This was the most basic test of the bacteria's ability to survive and produce virulence factors. Lastly, a mannose broth was used because mannose-6-phosphate is a precursor to many extracellular polysaccharide components including alginate, biofilm components, and LPS components (5). The results allowed us to determine if this factor alone was enough for alginate production or alternatively if the bacteria required other elements. The addition of other elements offered information about what the bacteria can use to increase their virulence. Calcium had previously been shown to induce excess extracellular polysaccharides in PA (10) and oxygen

has been shown to increase the amount of pyocyanin in culture (8). Since many factors appear to influence the production of extracellular polysaccharides in PA, it was hypothesized that the factors asparagine, citrate, glucose, mannose, KNO_3, and $CaCl_2$ chosen for this study would demonstrate various degrees of influence on slime production.

MATERIALS AND METHODS

To study the production of slime by PA, a simple, statistically reproducible procedure was previously developed. This procedure involved measuring the flow rate of the slime and all of its components as they passed through a device constructed from a funnel (top, outer diameter = 10cm; top, inner diameter = 9.3cm; cone height = 8cm; pipette insertion point = 1cm from base of cone), standard non-hardening modeling clay that can be purchased at any grocery store, and a Pasteur pipette (length = 22.2cm; top diameter = 0.65cm) (Fig. 2). First the clay was placed into the top of the funnel stalk and spread thinly where the Pasteur pipette would contact it. Next the pipette was fitted into the stalk of the funnel and sealed tightly using the clay. To ensure no leaks formed, the clay was naturally pushed up and was spread thinly onto the top/inner portion of the pipette. The entire apparatus was then placed into a ring stand for support and a petri dish was placed underneath to catch the slime that flowed through the device. At this stage the Pasteur pipette could not be changed out between trials because the clay lost its adhesive qualities when wet.

Preliminary experiments were performed by growing bacteria in petri dishes containing 20mL quantities of Muller Hinton (BBL Microbiology Systems, Cockeysville, MD) and Tryptic Soy broths (Bacto Difco Laboratories, Sparks, MD) at 35° C for times ranging from 48 to 52 hours to determine the reliability of the tests. The broths were inoculated with a stock culture of a cystic fibrosis strain of PA (Texas Tech Health Science Center strain 16) by touching a colony on a Tryptic Soy agar (TSA) (Acumedia Neogen Corp., Lansing, MI) plate using a sterile wire loop followed by introducing the loop into the broth and distributing the bacteria using a back and forth motion. The loop was flamed between each broth inoculation and the time of inoculation was recorded on the top of the

dish. Petri dish incubation was preferred over tube incubation because it allowed for increased oxygen exposure and physical surface contact. It also allowed for easier collection of the slime. Liquid media was necessary for transformation of the bacteria to a mucoid state and stimulation of slime production. After the incubation period the samples were removed from the incubator and negative controls (consisting of un-inoculated broth) were tested by pouring them into the apparatus, which was stoppered at the beginning of the experiment. The stopper was then removed and a timer was started while the liquid exited the apparatus. Once the liquid stopped flowing, even if a small amount of broth remained in the very end of the pipette, the timer was stopped and the time recorded. The experimental samples were tested in the same manner. To ensure all of the slime from the samples was tested, the contents of each petri dish were transferred into the funnel directly by pouring very slowly and then the bottom of the dish was scraped using a plastic disposable loop to collect all of the adherent biofilm. Once in the funnel, a plastic dowel was used to remove any air bubbles present by moving in an up-and-down motion allowing the liquid to fill the space. The test samples were alternated between broth types and were performed in the order of inoculation. The time of the test was also recorded to provide an accurate incubation time. The reliability was determined by comparing the difference from an inoculated broth and its negative control over 4 separate trials with 5 replicates each for a total of 20 samples. The range and average of these data ± the standard error was used to show relative similarities in the numbers and a randomized t-Test showed that the data did not differ from one test to another. To serve as another control, two types of broths were always incubated together including a broth previously tested and a new type of broth. This ensured that the results of the new broth were likely reliable as long as the previously tested broth acted similar to previous tests.

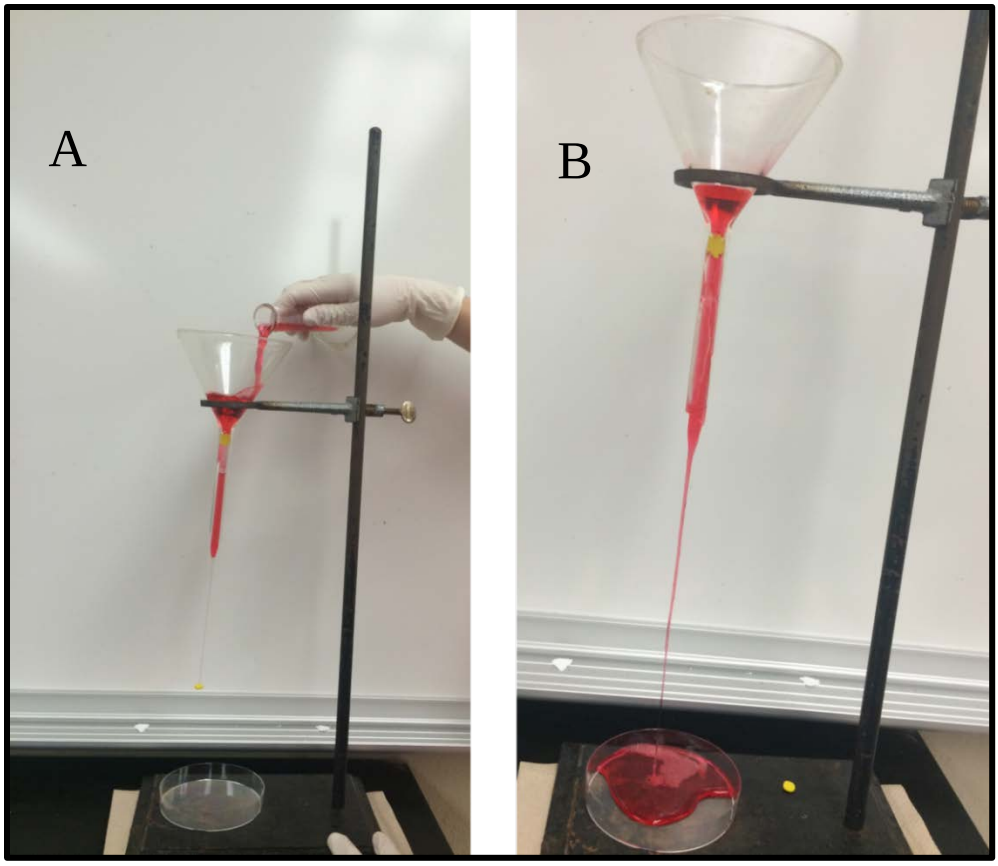


FIG 2 A) The broths, substituted for a red liquid in this photo for enhanced depiction, were poured into the apparatus first to ensure all air bubbles were removed. B) The stopper was removed and the liquid allowed to flow out while being timed

Once the reproducibility and reliability of the method was supported, further tests with minimal broths were designed. The minimal broths provided a single carbon source for the bacteria to offer information on which media components best promoted the production of virulence factors with particular focus on those that supported an abundance of alginate production. The broths used were a modified version of a minimal salt media used for testing the ability of *Pseudomonas aeruginosa* to grow with only one carbon source. The contents included 0.5g ammonium sulfate, 0.1g magnesium sulfate heptahydrate, 3g monopotassium phosphate, 6g sodium phosphate, one or three percent of a carbon source as mentioned before, and additional test salts or chemicals that might influence alginate production. All

media components were dissolved into one liter of distilled water before sterilization at 121° C for 20 minutes. Test chemicals added in certain experiments include 5g/ml of potassium nitrate or calcium chloride. To test the effects of mechanical manipulation some broths were incubated while being rotated in an incubator shaker (New Brunswick Scientific C25, Edison, NJ) at sixty revolutions per minute. Some of these broths were incubated for the standard 48 hours and then tested, while others were tested after another 48-hour stationary incubation. The latter tests were performed exactly the same as the preliminary experiments mentioned previously and the results were compared to the slime produced in the standard media. The latter served as a baseline control. After incubation the broths were checked for the presence of pyocyanin by illuminating the dish with a small Chauvet black light (Sunrise, FL) and looking for blue fluorescence.

All of these tests were designed to help determine which factors PA uses most in its production of alginate and other extracellular virulence factors. The program R (www.r-project.org) was used to determine the statistical significance of the tests. The results obtained yielded some insight into the synthesis of multiple components from a single carbon source and gave some insight into possible signaling pathways. The results might have practical implications as they relate to finding methods to negatively influence the ability of the organism to produce important virulence factors.

RESULTS

The reliability of the tests is shown by the reproducibility of result in the TSB and MHB broths when incubated for the same time. When subtracted from the negative controls both the TSB and MHB broths incubated at 48.5 hours were about 6.65±0.352 seconds longer than the controls ranging from 4.78 to 7.69 seconds and the test groups did not differ from each other significantly (permutational t-Test $p>0.05$).

The data from the single carbon sources were compared against their negative controls and each other. All of the data were normally distributed, however the homoscedasticity between groups was not equal (Levene's test: $F=2.0095$, $P<0.05$). Due to this observation, a randomized, permutational t-Test was used to determine significant differences in these data. The figures represent a total of 15 trials for asparagine, citrate, and glucose, 10 trials for KNO_3 addition to citrate and glucose, and 5 trials for the shake experiments.

The four different broths containing citrate, asparagine, glucose, or mannose as their single carbon source all showed differences in slime production by *Pseudomonas aeruginosa*. The citrate broths produced no fluorescence and lacked slime as evidenced by the finding that their flow through time was not statistically different from the negative controls (permutational t-Test, $p>0.05$) (Fig. 3). While the glucose broths produced no fluorescence or slime, their flow through time differed slightly from the negative control ($p<0.05$) due to the abundance of bacterial growth, low standard error values, and the sensitivity of the test (Fig. 3). Mannose broths apparently were unable to provide an appropriate carbon source for bacterial growth and consequently did not produce any viable bacteria. Asparagine broth in both 1% and 3% quantities showed a difference compared to

their negative controls and a much greater amount of slime than previously observed in any other broth (permutational t-Test, $p<0.05$) (Fig. 3). However there was a significant reduction in slime production from the 3% broth to the 1% broth. Due to the extreme level of slime production in the 3% asparagine, the 1% broths were used for all further tests so as to have a figure more comparable to the other single carbon sources. Asparagine broths also supported the greatest amount of fluorescence as compared to other broths (Fig. 4).

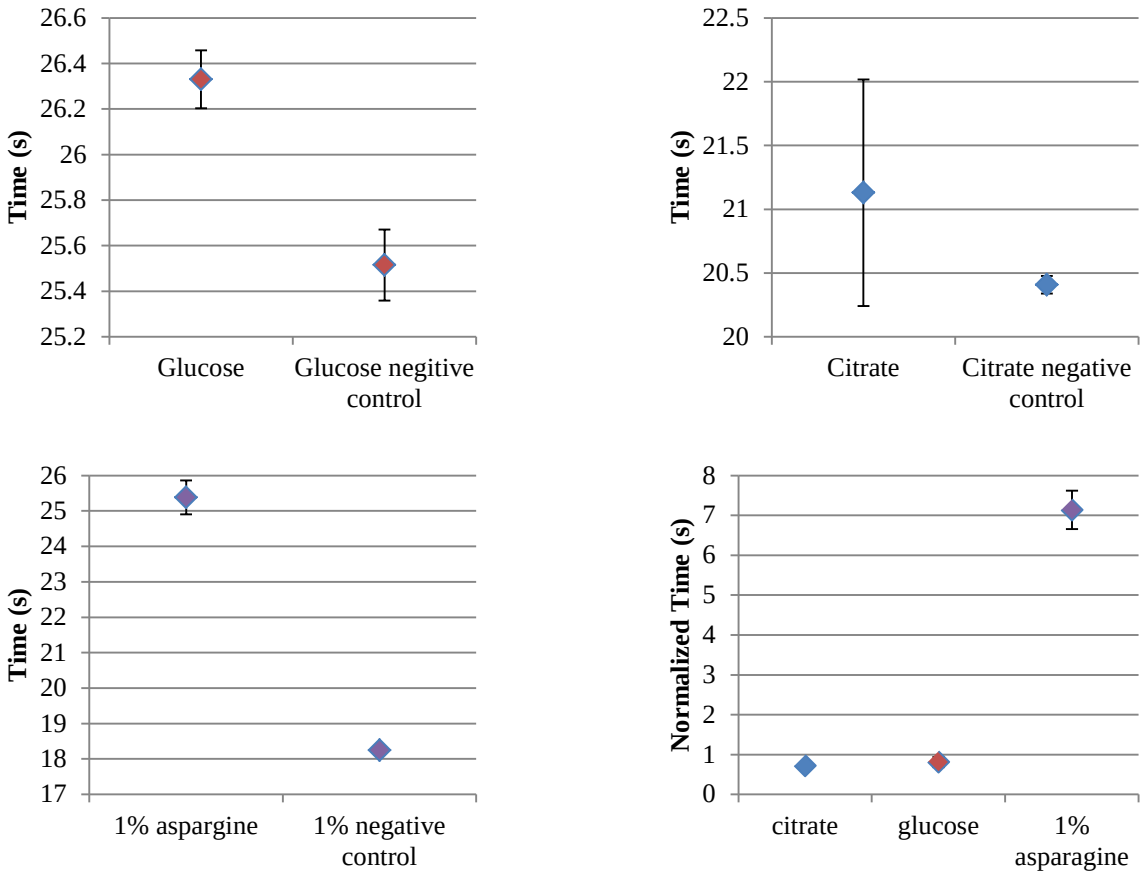


FIG 3 The average time for the broths inoculated with *Pseudomonas aeruginosa* to flow out of the apparatus versus its un-inoculated negative control differs significantly, except for the citrate broths. When the broths were normalized by subtracting the times of the negative control, one can observe the magnitude of difference in slime production by PA in asparagine when compared to the other broths

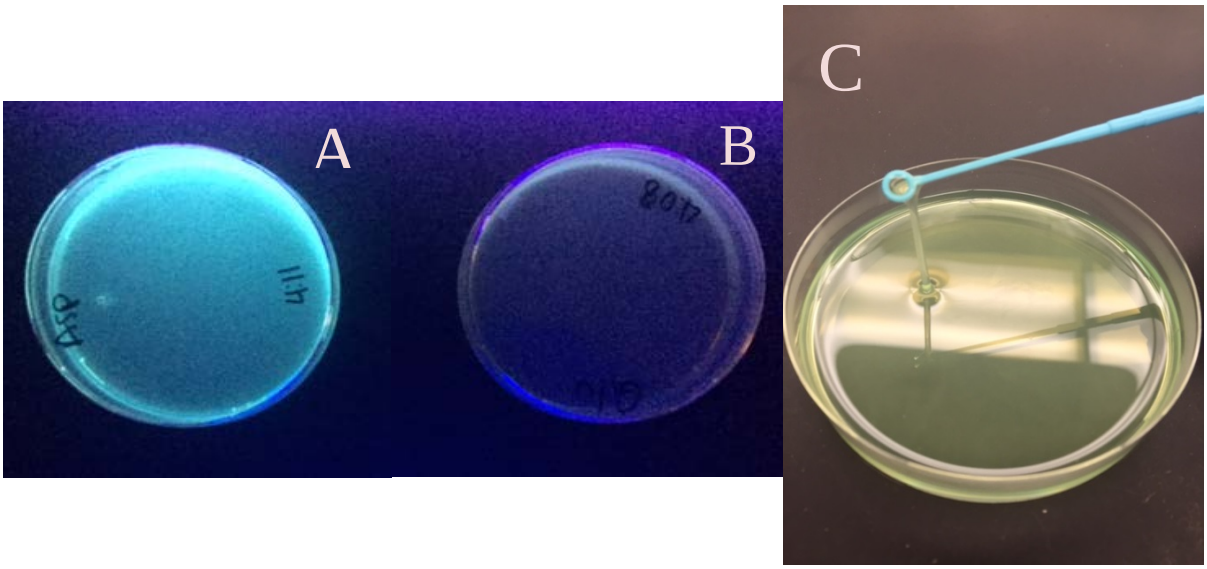
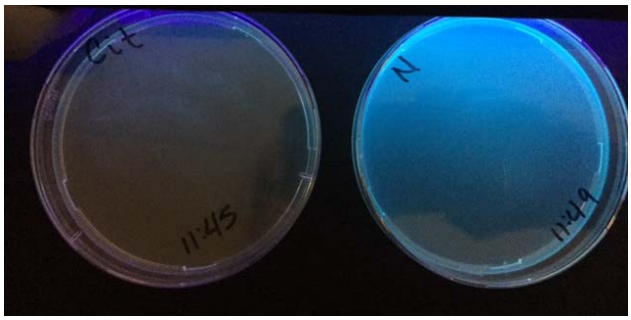


FIG 4 Pyocyanin production by PA was induced in the asparagine (A) but not the glucose broth (B) as seen by fluorescence of pyocyanin. (C) A loop demonstrates that the thick sticky slime can be lifted from the dish after 48 hour incubation in asparagine broth

With such great success in the asparagine broths, 1g per liter of potassium nitrate (KNO_3) was added to the other broths tested to determine if increasing the levels of inorganic nitrogen would have an effect on the alginate production in the other two broths. This salt did not change the glucose broth results other than the fact that when normalized by the negative control, the glucose broth with KNO_3 had a decreased average flow through time indicating less bacterial growth. The citrate broth responded to KNO_3 addition by producing slime as demonstrated by the significant difference in the time compared to the negative control (permutational t-Test, $p<0.05$) and fluoresced under UV light (Fig. 5).



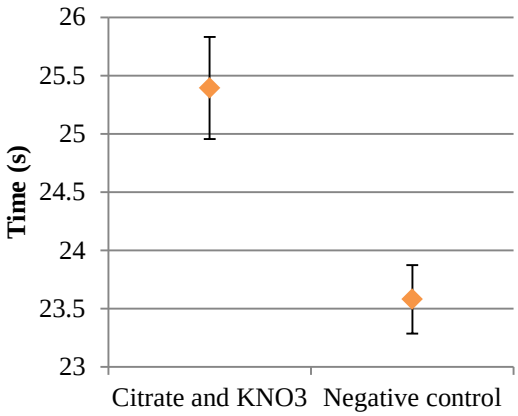
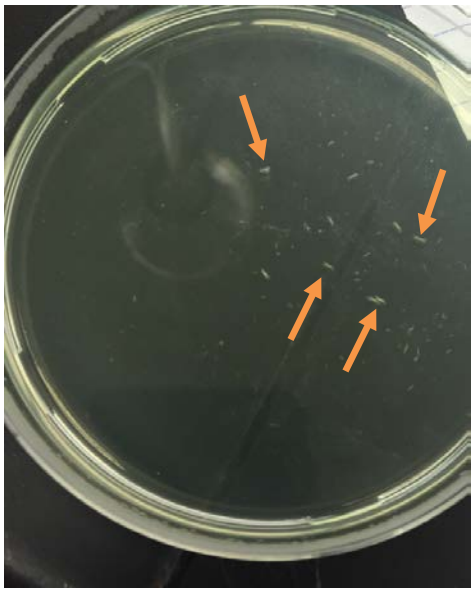


FIG 5 When potassium nitrate is added to the citrate broth, slime and pyocyanin production is induced in PA. The petri dish on the right contains citrate and KNO_3 while the broth on the left simply contains 3% citrate

The next set of experiments involved subjecting asparagine broths to mechanical manipulation. These broths did not yield any slime when compared to negative controls but did produce abundant fluorescence (permutational t-Test, $p>0.05$). The appearance of vegetative-like suspended colonies also appeared in these shaken broths (Fig. 6). The stationary incubation following the shake test yielded a slight amount of slime that differed from the control and did not differ from an asparagine broth incubated for four days (96 hours). When calcium chloride was added to the media, no apparent bacterial growth was visible.



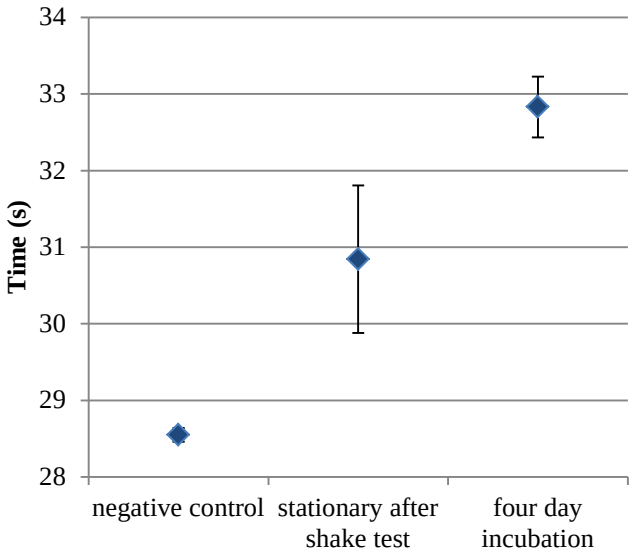


FIG 6 Vegetative colonies (arrows) formed while the asparagine broth shook during incubation. Both broths included on the graph incubated for a total of four days, one after 48 hours of shaking incubation and the other a four-day stationary incubation. Both differed from their negative control but not from each other.

DISCUSSION

The ability of *Pseudomonas aeruginosa* to change phenotypes from a single-celled state to a multi-cellular mucoid state requires the production of the appropriate amount of slime based on environmental cues. This slime protects the bacteria and is produced in abundance when the bacteria sense a less favorable environment. In addition, due to the great amount of proteins involved in the production, modification, and excretion of exopolysaccharides, alginate in particular (4,5,8), these bacteria use a great deal of energy and resources to produce these substances.

The bacteria grow well in broth with glucose as the carbon source because this sugar is abundant in energy and easy for the bacteria to metabolize. The abundance of available energy seems to promote single-celled logarithmic growth rather than alginate production. If the broths are incubated for longer than 48 hours the bacteria may sense a depletion of resources and transform into the mucoid state, producing alginate for greater protection.

Since asparagine and citrate are not as energy rich as glucose, it was predicted that protective extracellular polysaccharides might be formed more readily in these media because the growth environment becomes non-optimal more rapidly. This prediction was born out in the asparagine broths but not in the citrate broths. However, when KNO_3 was added to the citrate, the additional components seemed to provide signals that induced pyocyanin production that perhaps in turn enhanced alginate production. In addition this salt is known to increase the salinity of the broth, which has been previously shown to increase alginate production (7).

The environment inside the lungs of cystic fibrosis patients is not as favorable as one might initially predict. These patients produce abnormally thick mucus in their airways that

does not allow for ciliary removal of foreign materials (4). The bacteria must not only protect themselves from the immune system while surviving inside this thick mucus but this aerobic microbe must also contend with the patient's limitations in procuring and exchanging oxygen (3,4). To survive this environment and evade the immune system, one strategy that the bacteria use is to produce exopolysaccharides often in a structured and organized form called a biofilm. The lung environment is difficult to reproduce in the lab and the broths used in this study certainly do not reflect the environment of a cystic fibrosis lung. Nevertheless, it was observed that under less than perfect conditions and when given the necessary materials, these bacteria can readily undergo a change into the mucoid state and produce exopolysaccharides presumably including alginate. Previous studies suggest that certain stress response sigma factors acting as transcription factors can signal and regulate alginate production in these bacteria (7,10).

The shake test showed the propensity of the bacteria to produce exopolysaccharides even while unable to make successful continual contact with the bottom surface of the petri dish. The agitation of the broth promotes an increase of dissolved oxygen in the broth, which can stimulate increased bacterial growth and pyocyanin production (4). The broths produced no slime and did not differ from the negative controls as measured immediately after shaking, possibly due to the inability of the bacteria to contact a surface in order to produce a biofilm. However, when incubation was continued after shaking, the bacteria began to produce minimal amounts of slime, which differed slightly from the negative control. The asparagine broth incubated for four days produced significantly less slime than the typical two-day incubation, which might be attributed to bacterial death from diminished resources or perhaps reabsorption of the extracellular polysaccharides. The results indicated that 48

hours of incubation was optimal for slime measurement and that there was a drop off in the amount of slime present in the broth after a certain amount of resources were consumed by the bacteria. This could also be explained by the fact that PA has a tendency to reduce the expression of virulence factors once long term presence is established *in vivo* (4). In future studies, a one-day shake and one-day stationary incubation could better assay the effects of rapid, abundant pyocyanin production at a time the bacteria are still in an optimal growth phase and before the detrimental effects on the slime are initiated.

The calcium addition experiments resulted in no bacterial growth despite the conflicting results from Sarkisova et al. (10). Too much of the salt or possibly the mixture of this salt with other components in the media could have caused this.

This study raises many questions and provides a multitude of future ideas for studies concerning the production of extracellular polysaccharides in PA. For example, future studies might include subjecting the bacteria to stressors such as high salinity, lower and higher pH, different levels of UV radiation, and other manipulations. Such tests could help to determine whether stress is a key factor in inducing slime production in these bacteria.

To improve the present study a simple but specific test such as an enzyme linked immunosorbent assay (ELISA) might have been used to test for the presence of alginate in the broths to confirm its presence (7). Furthermore, molecular tests such as quantitative PCR could have allowed for observation of alginate gene expression in the differing single carbon broths to confirm the induction and subsequent production of this substance.

Studying and discovering the mechanisms and signals that induce the production of life threatening virulence factors in these bacteria will perhaps one day allow for the production of a drug that inhibits lung colonization by them. The ultimate goal is to help

cystic fibrosis and burn patients from ever becoming infected in the first place. Knowing which factors influence production of virulence factors in these bacteria might someday translate into an effective means of preventing life-threatening infections and thereby reduce not only the healthcare costs of these patients but also reduce the chances of encouraging microbial drug resistance. These latter two goals have general application to society as a whole.

LITERATURE CITED

1. **Antoniou S, Elston C.** 2016. Cystic fibrosis. Medicine. **44:**321-325.
2. **Van Gool K, Norman R, Delatycki MB, Hall J, Massie J.** 2013. Understanding the costs of care for cystic fibrosis: an analysis by age and health state. Val. in Health. **16:**345-355
3. **Winstanley C, O'Brian S, Brockhurst MA.** 2016. *Pseudomonas aeruginosa* evolutionary adaptation and diversification in cystic fibrosis chronic lung infections. Trends in Microbiol. **24:**327-337
4. **Rada B, Leto TL.** 2013. Pyocyanin effects on respiratory epithelium: relevance in *Pseudomonas aeruginosa* airway infections. Trends in Microbiol. **21:**73-81.
5. **Ma L, Wang J, Wang S, Anderson EM, Lam JS, Parsek MR, Wozniak DJ.** 2012. Synthesis of multiple *Pseudomonas aeruginosa* biofilm matrix exopolysaccharide is post-transcriptionally regulated. Environ. Microbiol. **14:**1995-2005.
6. **Tan J, Rouse SL, Li D, Pye VE, Vogeley L, Brinth AR, Arnaout TE, Whitney JC, Howell PL, Sansom MSP, Caffrey M.** 2014. A conformational landscape for alginate secretion across the outer membrane of *Pseudomonas aeruginosa*. Acta. Cryst. **70:**2054-2068.
7. **Davies DG, Chakrabarty AM, Geesey GG.** 1993. Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **59:**1181-1186.
8. **Maleki S, Almaas E, Zotchev S, Valla S, Ertesvag H.** 2016. Alginate biosynthesis factories in *Pseudomonas fluorescens*: localization and correlation with alginate production levels. Appl. Environ. Microbiol. **82:**1227-1236.

9. **Moradali MF, Donati I, Sims IM, Ghods S, Rehm BHA.** 2015. Alginate polymerization and modification are linked in *Pseudomonas aeruginosa.* Mbio.asm.org **6:**e00453-15.

10. **Sarkisova S, Patrauchan MA, Berglund D, Nivens DE, Franklin MJ.** 2005. Calcium-induced virulence factors associated with the extracellular matrix of mucoid *Pseudomonas aeruginosa* biofilms. J. Bacteriol. **187:**4327-4337.

VITA

Chelbee Farnen was born in San Antonio, Texas, and lived most of her childhood in Austin and Marble Falls, Texas. She graduated top 10% of her class from Marble Falls High School and decided to attend Angelo State University to study biology. At ASU she was accepted to the Honors Program where she excelled and took advantage of many opportunities the program offered. The summer after her third year Chelbee received the Alvin New Family Honors Enhancement Grant to study abroad at the Imperial College in London taking a course entitled “The Revolutions in Biomedicine”. This course along with her two years of undergraduate research confirmed her love for biomedical research.

After earning her bachelors of science in biology from ASU Chelbee will attend the University of Texas Health Science Center in San Antonio. Here she will pursue a PhD in Integrated Biomedical Sciences with emphasis in “Infection, Inflammation, and Immunity” and will hopefully work in a lab to perform research in microbiology. Chelbee one day hopes to teach at the university level while continuing her research.